

# Effect of Broccoli Flower Extract (*Brassica oleracea* L. var. *italica* Plenck) on Inhibition of Photoaging Viewed from Matrix Metalloproteinase-1 Expression in Human Skin Fibroblast

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## Abstract

**Objective :** Ultraviolet irradiation is the major external factors that cause skin photoaging. Ultraviolet B (UVB) irradiation induces the production of matrix metalloproteinase (MMP) by activating cellular signaling transduction pathways, which are responsible for the degradation of collagen. The imbalance between collagen degradation and synthesis play a major role in the formation of wrinkle of photoaging. Broccoli (*Brassica oleracea* L. var. *italica* Plenck) is a cruciferae group vegetables which has a great amount of antioxidant.

The aim of this study is to prove whether broccoli flower extract (BFE) as an effective antiphotaging agent by a MMP-1 inhibitor action in vitro. **Methods :** An experimental (in vitro) study had been conducted using UVB irradiated primary human skin fibroblast culture. We studied the MMP-1 inhibitory effects of BFE on photoaging. The quantification of MMP-1 mRNA expression was done by Realtime RT-PCR while the protein expression assay was done by ELISA. **Results :** Pretreatment with BFE decrease MMP-1 expression both at MMP-1 mRNA and MMP-1 protein expression. There were significant differences of mean value of MMP-1 mRNA and MMP-1 protein expression between every group based on irradiation dose ( $p < 0.05$ ) and BFE concentration ( $p < 0.05$ ). There were also interaction between irradiation dose and BFE concentration ( $p < 0.05$ ). We found there were negative correlation between BFE concentration and either MMP-1 mRNA or protein expression. There was positive correlation between MMP-1 at mRNA level with protein level. **Conclusion :** BFE prevents UVB-induced MMP-1 expression both at mRNA and protein level, therefore BFE might be used as a potential agent for skin photoaging.

**Keywords :** broccoli flower extract, photoaging, matrix metalloproteinase-1, human skin fibroblast

## 1. Introduction

Skin change is one of the most prominent signs of aging. Skin can age in two ways, chronologic aging and extrinsic aging which occurs as a consequence of exposure to environmental factors (Jenkins, 2000). One of the most important external factor is sunlight irradiation which causes skin photoaging (Yaar & Gilchrist, 2007). Alterations and deficiencies of collagen, the major structural component of the skin, have been suggested to be a cause of the wrinkle of photoaging and naturally aged skin. The expression of matrix metalloproteinase (MMP)-1 and the down regulation of type 1 procollagen synthesis play a major role in the process of photoaging (Rittie & Fisher, 2002 ; Fowler, 2003).

It has been wellknown that chronic exposure of human skin to UVB irradiation results in photoaging and induces the production of MMPs (Brennan, et al., 2003). Recent studies have shown that hairless mice exposed to UVB developed wrinkled skin and significantly enhance MMP-1 mRNA expression (Takema, et al., 1999). In the absence of a perfect repair, MMP-1 mediated collagen damage is accumulated with each successive UV exposure. However it has shown that the inhibition of MMP-1 activities by a specific MMP inhibitor suppresses UVB-induced wrinkle formation (Frei, 2004).

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) is a cruciferae group vegetables which has a great amount of antioxidant (Fahey & Talalay, 1999). It has been proved in dermatology as antiinflammatoric (Talalay, 2007) and antimutagenic agents (Kern, et al., 2007).

## 2. Materials and methods

### 2.1. Plant material

Broccoli flowers (*Brassica oleracea* L. var. *italica* Plenck) were collected from a broccoli field at Berastagi, North Sumatera, Indonesia. After processing to simplicia, it extracted in 96% ethanol in the laboratory of Faculty of Pharmacy University of Sumatera Utara.

### 2.2. Cell culture

The normal human fibroblast cells were aseptically isolated from preputium circumcised skin. After the

epidermis and dermis were separated mechanically, the dermis was minced and attached on the surface of tissue culture flask. The cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (GIBCO, Grand Island, NY USA). After 3 passages the fibroblast were used for the experiment.

### 2.3. Ultraviolet irradiation

The UV light source originated from a Philips TL 20 W(12 RS fluorescent sun lamp) with an emission spectrum of 285-350 nm (peak at 310-315 nm). The cells were then exposed to 50 and 100 mJ/cm<sup>2</sup> dose of UVB light.

### 2.4. Broccoli Flower Extract (BFE) treatments

BFE was dissolved in DMEM. The BFE concentration that use for treatment comprise of 25, 50 and 100 µg/ml. For treatment, the cells were maintained in culture media without FBS overnight, followed by treatment with BFE for 24 hour. The cells were rinsed twice with phosphate buffer saline (PBS) and UVB irradiation exposure were performed under a thin layer of PBS. Immediately after irradiation, the cells were incubated in serum-free fresh culture media containing BFE. Every treatment was done triple.

### 2.5. RNA extraction and Realtime Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

To assay the MMP-1 mRNA, cell was isolated using Allprep DNA/RNA/protein (Qiagen) kit. RNA concentration was quantified by spectrophotometer at 260 nm and the purity was determined using A 260/280 ratio. All samples were reverse transcribed by Transcriptor First Strand cDNA synthesis kit (Roche). The RT-PCR assay was specifically quantify mRNA level. In all assays cDNA was amplified using a standardized programme (30 seconds denaturing step at 94°C, 30 seconds annealing step at 60°C, 30 seconds elongated step at 72°C). The expression on MMP-1 mRNA was determined by realtime PCR using Light cycler 1,2 (Roche) and the SYBR Green (Applied Biosystem Roster City, CA). The primer were human MMP-1 sense primer, 5' – AAG CGT GTG ACA GTA AGC-TA 3'; anti sense primer, 5' AAC CGG ACT TCA TCT CTG-3'. The absolute quantification of primers had been done based on fluorescence signal from samples.

### 2.6. ELISA

The supernatant from the culture was collected and the MMP-1 protein expression was quantified by Human MMP-1 ELISA kit ( Boster Biological technology Ltd, China ) at 450 nm using a microplate reader.

### 2.7. Statistical Analysis

Data were expressed as the mean value and analyzed by analysis of variance (Anova) 2 ways. Statistical significance was set a prior at p<0.05. Correlation between BFE concentration and MMP-1 expression either at mRNA or protein level were analyzed by Spearman's correlation test. Correlation between MMP-1 at mRNA level and protein level was analyzed by Spearman's correlation test.

## 3. Result

### 3.1. Effect of BFE on UVB-induced MMP-1 mRNA expression

To study the effect of BFE on UVB-induced MMP-1 mRNA expressions, we performed Realtime RT-PCR in human dermal fibroblast. It revealed that UVB irradiation either at 50 or 100 mJ/cm<sup>2</sup> increased the level of MMP-1 and the BFE blocked UVB-induced up regulating of MMP-1 (Fig.1). Anova two ways showed there were significant differences of mean value of MMP-1 mRNA between every group based on UVB irradiation dose (p<0,05) and BFE concentration (p<0,05) (Table 1). Continued by multiple comparison test (LSD) showed significant differences (p<0,05) between every group based on irradiation dose and BFE concentration. These result indicated that BFE is a potent inhibitor of MMP-1 on photoaging in UVB-irradiated human skin fibroblast.

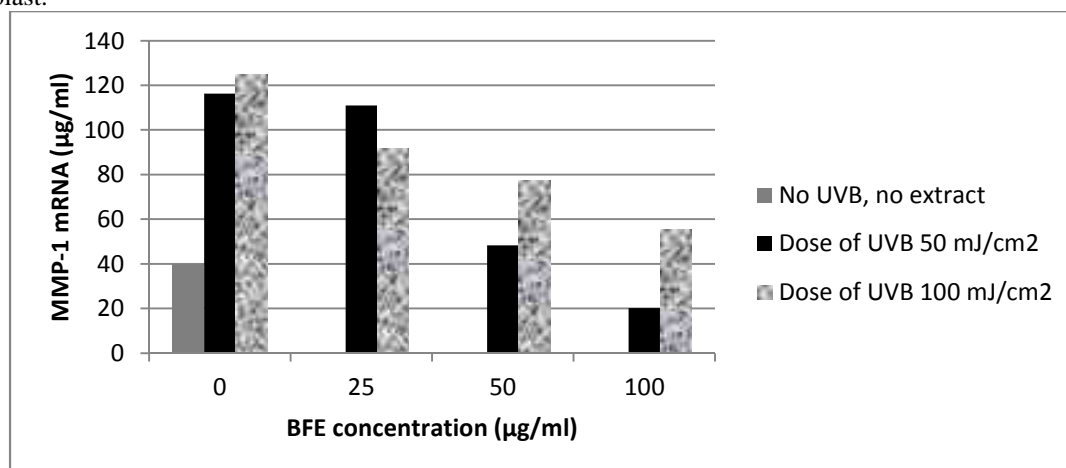


Figure 1. Effect of BFE on UVB-induced MMP-1 mRNA expression

Table 1. MMP-1 mRNA expression based on irradiation dose and extract concentration with Anova two ways test

Sources	Sum of squares	Df	Mean square	F	P
Irradiation dose	1086,760	1	1086,76	28767,187	0,0001*
Extract concentration	25016,831	3	8338,944	220736,7	0,0001*
Dose/concentration	2719,058	3	906,353	23991,688	0,0001*
Error	0,680	18	0,038		0,0001*
Total	189648,830	27			0,0001*
Corrected Total	33225,274	26			0,0001*

\*statistically significant

### 3.2. Effect of BFE on UVB-induced MMP-1 protein expression

To study the inhibitory effect of BFE on UVB-induced MMP-1 at the protein level, ELISA analysis was performed. UVB-induced MMP-1 protein expression was significantly inhibited by the action of BFE. Treatment of BFE with 25, 50 and 100 µg/ml inhibited MMP-1 protein expression in UVB-irradiated human skin fibroblast (Fig. 2). By Anova two ways analysis we found there were significant differences of mean value of MMP-1 protein expression between every group based on UVB irradiation dose ( $p < 0.05$ ) and BFE concentration ( $p < 0.05$ ) (Table 2). Continued by multiple comparison test (LSD) there were significant differences ( $p < 0.05$ ) between every group based on irradiation dose and BFE concentration. These result indicated that BFE down regulated MMP-1 expression on photoaging in UVB irradiated human skin fibroblast culture.

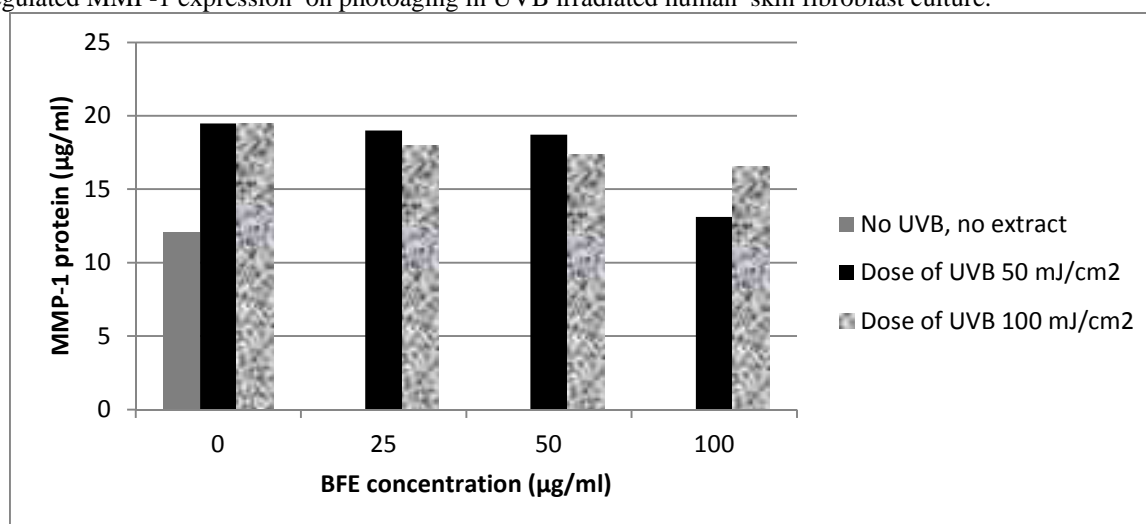


Figure 2. Effect of BFE on UVB-induced MMP-1 protein expression

Table 2. MMP-1 protein expression based on irradiation dose and extract concentration with Anova two ways test

Sources	Sum of squares	Df	Mean square	F	P
Irradiation dose	0,468	1	0,468	114912,7	0,0001*
Extract concentration	72,631	3	24,210	5942,502	0,0001*
Dose/concentration	22,006	3	7,335	1800,464	0,0001*
Error	$7,33 \times 10^{-5}$	18	$4,07 \times 10^{-6}$		
Total	805,667	27			
Corrected Total	180,389	26			

\*statistically significant

### 3.3. Correlation between BFE concentration with MMP-1 mRNA and MMP-1 protein expression

By Spearman's correlation test we found there were significant negative correlation between BFE concentration with MMP-1 mRNA expression at 50 mJ/cm² UVB irradiation dose ( $r = -0.972$ ,  $p < 0.01$ ) and at 100 mJ/cm² UVB irradiation dose ( $r = -0.972$ ,  $p < 0.01$ ), as well as BFE concentration and MMP-1 protein expression at 50 mJ/cm² UVB irradiation dose ( $r = -0.973$ ,  $p < 0.01$ ) and at 100 mJ/cm² UVB irradiation dose ( $r = -0.972$ ,  $p < 0.01$ ) (Table 3).

Table 3. Correlation between BFE concentration with MMP-1 mRNA and MMP-1 protein expression

		n	r	p
Extract concentration	MMP-1 mRNA (50)	12	-0,972	0,0001
	MMP-1 protein (50)	12	-0,973	0,0001
	MMP-1 mRNA (100)	12	-0,972	0,0001
	MMP-1 protein (100)	12	-0,972	0,0001

Spearman's correlation test; Correlation is significant at the 0,01 level (2-tailed); 50: UVB irradiation dose 50 mJ/cm<sup>2</sup>; 100: UVB irradiation dose 100 mJ/cm<sup>2</sup>; r: correlation coefficient

### 3.4. Correlation between MMP-1 mRNA level with protein level

By Spearman's correlation test we found there were significant positive correlation between MMP-1 mRNA with protein level at 50 mJ/cm<sup>2</sup> UVB irradiation dose (r=0.911, p=0.0001) and 100 mJ/cm<sup>2</sup> UVB irradiation dose (r=0.972, p=0.0001) (Table 4). These result indicated BFE inhibit UVB-induced MMP-1 at the primary regulation (mRNA level) and also at the extracellular protein regulation step.

Table 4. Correlation between MMP-1 mRNA level with protein level

mRNA	Protein	n	r	p
MMP-1 (50)	MMP-1 (50)	12	0,911	0,0001
MMP-1 (100)	MMP-1 (100)	12	0,972	0,0001

Spearman's correlation test; Correlation is significant at the 0,01 level (2-tailed); 50 : UVB irradiation dose 50 mJ/cm<sup>2</sup>; 100 : UVB irradiation dose 100 mJ/cm<sup>2</sup>; r : correlation coefficient

## 4. Discussion

Recent evidence indicates that chronologically aged and UV-irradiated skin share important molecular features including altered signal transduction pathways that promote MMP expression, decrease procollagen synthesis, and connective tissue damage (Brenneisen, et al., 2002; Choi, et al., 2006; Fisher, et al., 2009; Varani, et al., 2000).

It is known that the mechanism for skin photoaging is due to the production of reactive oxygen species (ROS) induced by UV irradiation. The activation of ROS leads to a series of signal transduction and stimulate the gene transcription of matrix-degrading enzymes, such as MMP-1 (collagenase) (Yamamoto, 2001), which is the major collagenolytic enzyme responsible for collagen damage in UV-irradiated human skin (Brennan, et al., 2003)

The increasing of age will induce the rise up of MMP levels and the decrease of collagen synthesis (Varani, et al., 2000). Hence, the development of MMP inhibitors is considered to be a promising strategy for photoaging (Hu, et al., 2000). In recent years, the development of compounds with MMP inhibition activities from natural plants has received a great deal of attention (Moon, et al., 2005; Choi, et al., 2007; Kim, et al., 2007; Hsu & Chiang, 2009). The antioxidant capable to inhibit the mitogen activated protein kinase (MAPK) pathway that produce MMP-1, therefore premature aging induced by UVB irradiation may be inhibited (Frei, 2004). Broccoli flower extract (*Brassica oleracea* L. var. *italica* Plenck) contains of multiple antioxidants and vitamins such as sulforaphane, indole, vitamin A C, E, beta carotene, quercetine, kaempferol, glutathione and selenium (Ipteknet, 2005; Jeffery & Araya, 2009).

We studied the MMP-1 inhibitory effect of BFE as a new potential anti-photoaging substance by various in vitro experiments. UVB can induce MMP-1 expression as a function of dose, similarly as shown in our study either at UVB irradiation dose of 50 or 100 mJ/cm<sup>2</sup>. The time after UV exposure in vitro directly determines the extent of damage that is inflicted on MMP-1 expression (Brennan, et al., 2003). MMP-1 expression increases both as a function of time and dose in cultured cells in response to UV exposure (Moon, et al., 2009; Moon, et al., 2008). We found that pretreatment with 25, 50 and 100 µg/ml BFE concentration, inhibited UVB-induced MMP-1 expression either at mRNA or protein level compared to the UVB-only irradiated group. Pretreatment with BFE decrease MMP-1 expression both at mRNA and protein level. There were interaction between UVB irradiation dose and BFE concentration to the expression of MMP-1. It means that UVB irradiation dose and BFE concentration together influence the mean value of MMP-1 expression both at mRNA and protein level. We also found there were negative correlation between BFE concentration with MMP-1 mRNA and MMP-1 protein expression, thereby the increasing of BFE concentration leads the MMP-1 expression decline. There were positive correlation between MMP-1 expression at mRNA level and protein level at 50 mJ/cm<sup>2</sup> and 100 mJ/cm<sup>2</sup> UVB irradiation.

This study demonstrates the inhibitory effect of BFE on the MMP-1 expression via mRNA and protein assay in skin photoaging process that induced by UVB in vitro. Therefore, we suggest that BFE should be viewed as a potential therapeutic agent for preventing and treating skin photoaging in the near future.

## 5. Conclusion

BFE had MMP-1 inhibitory effects either at mRNA or protein level on the process of photoaging in UVB

irradiated human fibroblast culture. Therefore BFE has been proved as an antiphotoreactive agent by MMP inhibitor action in cellular as well as molecular level in vitro.

Finally it is suggested that further investigation on BFE involving animal and human subjects should be carried out.

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